DEVELOPMENT OF ANTI-INFLAMMATORY SOAP ENHANCED WITH GREEN COFFEE BEAN OILS

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***Abstract*— Green coffee contains a large quantity and variety of polyphenols and flavonoids. The composition of the polyphenols in coffee, due to the formation of compounds generated by Maillard reaction, which can have anti-inflammatory potential. Inflammatory skin illnesses are the most well-known issue in dermatology. This type of illness has been known one of the biggest illness in Malaysia for the past few years. Thus, in this study shows the reliable on Green Coffee Bean oils extracted using Soxhlet extraction to be applied in commercial soap for the anti-inflammatory effect. A simple method for the determination of chlorogenic acids content in green coffee bean was reported. The method was based on the use of UV/Vis absorption. It is relevant that the quantification of and chlorogenic acids was performed without their preliminary chemical separation despite their spectral overlap in the range 200–400 nm. Themolar extinction coefficients chlorogenic acid in ethanol solution was calculated by using the chemical standards; the estimated values were ε(330nm) = 27025 ± 190M−1 cm−1 for chlorogenic acids molecules. The anti-inflammatory effects extracts of GCB applied in soap was investigated in animal models and using a Carrageenan-induced rat paw oedema. In the formalin test the extracts reduced licking activity.**

Keywords*— Green Coffee Bean, Chlorogenic Acids, Soap, Anti- Inflammatory, Uv-Vis.

I. INTRODUCTION

Coffee has been enjoyed as a drink by millions of people worldwide for at least 1000 years. Due to their unique taste and flavor, coffee brews are among the most consumed beverages in the world [1]. Coffee is consumed for social engagements, leisure, enhancement of work performance and well-being. Green coffee bean is the major source of polyphenols, in particular, chlorogenic acids (CGAs), in the human diet. Green coffee beans are high in CGAs; their contents are 3.5–7.5% (w/w dry matter) for Coffea arabica and 7.0–14.0% (w/w dry matter) for Coffea canephora [2].

The nomenclature of CGAs is based on the IUPAC numbering system (1976), and 5-caffeoylquinic acid (5-CQA) is generally called chlorogenic acid. Thirty-four kinds of CGAs have been reported in green coffee beans. Chlorogenic acid is a polyphenol compound found in coffee beans and different types of coffee, including green coffee. It is also found in food sources such as apples, pears, eggplant, blueberries, tomatoes, strawberries, and potatoes [3]. From previous research, there are results on antioxidant and anti – inflammatory effects from Chlorogenic Acid. This effect is from the inhibition of the production on cytokines on skin tissues. Thus, prevent and meditate the inflammation.

Inflammatory skin illnesses are the most well-known issue in dermatology. They come in numerous structures, from infrequent rashes joined by skin tingling and redness, to perpetual conditions, for example, (dermatitis), rosacea, seborrheic dermatitis, and psoriasis. Skin irritation can be portrayed as intense or ceaseless. Intense aggravation can result from presentation to UV radiation (UVR), ionizing radiation, allergens, or to contact with synthetic aggravations (cleansers, hair colors, and so on.). This irritation is enduring and can cause critical and genuine tissue devastation. Fiery skin conditions influence more than 7 million Malaysian who every year spend over RM 1 million to treat their manifestations [4].

Anti- inflammatory agents such as Phenolic Compound that consist of Chlorogenic Acid are needed for human skin and body to cure the inflammation reaction. Phenolics are a heterogenic group of compounds derived from the secondary metabolism of plants. Structurally, phenolic compounds have at least one aromatic ring to which one or more hydroxyl groups are bonded to aromatic or aliphatic structures. Phenolic compounds can be grouped into flavonoids and non-flavonoids [5].

Topical steroids are one of manufactured medications that used to contemplate skin aggravation. Topical steroids are a simple method to treat the biggest and most open organ that is the skin. They are today viewed as the pillar treatment of numerous dermatologic conditions since they can be connected specifically to the influenced region and have less reactions contrasted with oral steroids. Topical steroids are accessible in treatments, creams, moisturizers, gels, and even powders. While salves are the most strong of the five, others might be increasingly fitting to specific conditions (Heather Brannon, 2018). There are few drawbacks and reactions on accepting topical steroids as calming operator contrast with characteristic enemy of – incendiary specialist yet there are still little measure of research on normal mitigating operator.

Accordingly of these issues, this examination propose to research the utilization of against – provocative cleanser by utilizing the extraction of oil from Green Coffee Bean. Studies demonstrate chlorogenic acid, polyphenols, caffien and flavonoids from the oil substance are concentrated with cancer prevention agent. Concentrates on cell reinforcement are one of the properties to lessen aggravation had been demonstrated previously [10]. Thus, regarding on the inflammation problems, our study propose to develop anti- inflammatory actor in soap enhanced with Green Coffee Bean Oil.

II. METHODOLOGY

A. Materials and chemicals

Green coffee (C. arabica L.) cv. Yellow Bourbon, sieve 16/18, without imperfections, harvested in 2018, and was kindly supplied by F Marketing (Selangor, Malaysia). The beans were packed in non-permeable polyethylene bags hermetically sealed under

vacuum. Afterwards the extracts were prepared at room temperature (25ºC).

Reference standards that is 5-caffeoylquinic acid (chlorogenic acid; CGA), Indomethacin, Carrageenan, Formalin, Sodium Hydroxide (NaOH) with purity of 99%, Sodium Chloride (NaCl) for Saline solution Coconut Oil are going to be obtained from Sigma Chemical Co., St. Louis, MO, USA. Extraction solvent, ethanol was purchased from Chemiz (M) Sdn. Bhd (Selangor, Malaysia).

B. Preparation of Extraction

The sample for this study is going to be fresh unroasted Green Coffee Beans. The samples are going to be washed thoroughly to remove any impurities or dirties. Then, the samples are going to be grinded to become powder or small particle sizes as it will increase the surface area contact on the Green Coffee Beans to be extracted. The powdered Green Coffee Beans will be weighted to approximately 20g for every sample that will be used.

C. Extraction of Green Coffee Bean

Powdered green coffee beans of 20g were loaded into Soxhlet extraction thimble. The extraction is going to be controlled by the temperature of between 80 ºC – 200 ºC. The powdered green coffee beans were extracted using varied amount of Ethanol in the range of 160 – 300 mL [7]. The extraction was carried out in 6 hours with few amounts of cycles. The mixture of ethanol (solvent) and Green Coffee Bean Oil extracted were collected at the bottom flask. The ethanol-oil mixture was subsequently evaporated from the extracted oil in a rotary evaporator [8]. The oil sample were stored into the vial with freezer temperature condition.

D. Total Chlorogenic Acid Content Analysis

Different concentrations of reference and test solutions are going to be detected under a UV-Visible Spectrophotometer (Perkin Elmer Lambda 750) at the range from 200 to 400 nm by using halogen and deuterium sources for the visible and ultraviolet radiations, respectively. After the basic preparation, the samples appropriately diluted in Ethanol inn order to make spectroscopic measurement. The contribution of the solvent absorption was subtracted from the obtained raw spectra. All measurements were made in triplicate at the controlled temperature of 25 ºC using a quartz square cuvette with 1 cm optical path.

The determination of the molar extinction coefficients of CGA at 330 nm was realized by applying the well-known Lambert-Beer law, by fitting linearly the dependence of the absorbance versus the sample concentration. The error associated with the concentration was the standard deviation obtained from triplicate measurements.

E. Formulation of Soap enhanced with Green Coffee Bean's Oil

Coconut oils (25 g) was heated and stirred with by using magnetic stirrer which 550 rpm at temperature 80 ºC for 5 minutes. Sodium Hydroxide (NaOH) (5 g) was added into the heated coconut oils. The NaOH was diluted in 15 mL of distilled water. Stir the mixture until trance. Trance is a condition where the soap has been formed signed as the end of the mixing process which is also the soap mixture begins thickening. When it touches with a spoon, then scoop in few seconds still lasting scars. The difference between the temperature of NaOH solution with the mixture of oil are not more than 50 ºC. After that, the temperature was lowered to 40 ºC and the green coffee bean oils extracted are going to be added for 0.5g of the Green Coffee Bean oils that is 5%. The mixture was poured into a mould, after 10 minutes covered with plastic then storage at ambient temperature for 24 hours until the soap hardens [9].

F. Anti – Inflammatory Soap Activity Analysis

Adult male rats (180-220 g) and adult male mice (25-35 g), are going to be housed under controlled light and temperature conditions of 23 ºC with access of food and water. At the end of the experiment, rats are going to be euthanised using an overdose of anaesthetic. All experiments are going to be conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals.

A formalin solution (5% in saline ; 20 µl l per paw) is going to be injected into the hind paw plantar surface and the animals are individually placed in transparent observation chambers. The animals are going to be treated with reference drug that is indomethacin and administered 1 hour prior to formalin injection (n = 2 per group). The soap enhanced by green coffee beans oil are going to be placed along the floor of the chambers and also administered 1 h prior to formalin injection (n = 2 per group). The time spent licking the injected paw is going to be recorded and expressed as the total licking time during the early phase (0-5 min) and late phase (20-30 min) after formalin injection [10].

Paw oedema is going to be measured with a plethysmometer, which consists of the immersion of the animals paws in a vat, containing transducer ionic solution, is going to be utilised to measure the edematogenic process and the volume of the paw was established directly in ml, through a digital system [11]. The basal volume of the right hind paw is going to be determined before the administration of any drug. After determination of the basal volume, the animals (n = 3 per group) are going to be divided into experimental groups in such a way that the mean volumes of the different groups were similar. The indomethacin (10 mg/ kg) are going to be orally administered 1 hour before the injection of carrageenan (1 mg). The soap enhanced by green coffee bean oils are going to be placed along the floor of the chambers and administered 1 hour before the injection of carrageenan (1 mg). The paw volume is going to be measured 1, 2, 3 and 4 hours after injection of the inflammatory stimulus [10]

III. RESULTS AND DISCUSSION

A. Chemical Standard Characterization

Fig 1 shows the optimization of CGA concentration relation with Temperature and Solvent Volume using Soxhlet extraction. From this optimization, it shown that, that optimum value is when at 200ºC using 300 ml of solvent that is ethanol. Corroborating our result, Khoddami Ali demonstrated that the increment of solvent volume will increase the extraction yield for solvent extraction (Soxhlet extraction) [17].

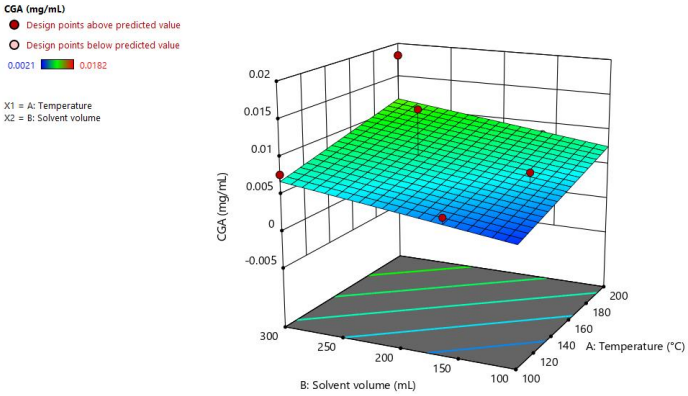

Fig 1: Response surface plots showing the influence of Temperature and Solvent Volume on the CGA (mg/ml)

Fig 2 shows the UV-Vis absorption spectra of CGA measured in distilled water in the wavelength regions of 200-500 nm at room temperature. In this region, CGA has two peak points; that is the first peak point is being at 217 nm with shoulder at 241 nm and the second maximum was at 330 nm with shoulder at 300 nm and the minimum point was at 272 nm. The maximum point at 330 nm was the highest point corresponding to the HOMO-LOMO transition presents mainly a $\pi\pi^*$ character with electron density localization on the benzene ring and carbon chain and this large absorbance seem to be promising to improve the sensitivity for CGA determination in coffee beans [12].

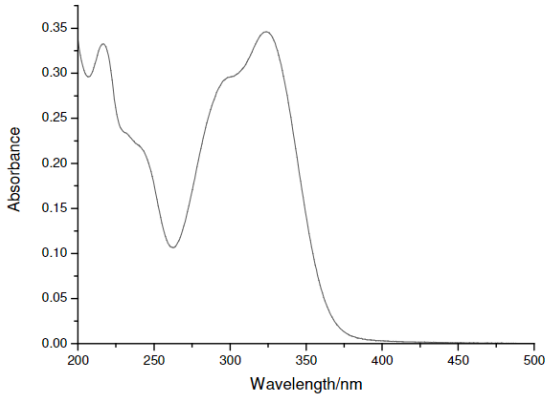

Fig 2: UV/Vis absorption spectra of CGA chemical standard

CGA spectra, was realized at dilutions. The molar extinction coefficient values, at 330 nm for CGA, was calculated by fitting the linear dependence of absorbance amplitude by the sample concentration values. The resulting graphs were reported in Fig 2. The obtained values were (330nm) = 27025 ± 190M−1 cm−1 for CGA. These values were found in good agreement with values early obtained using different solvents and reported in the literature.

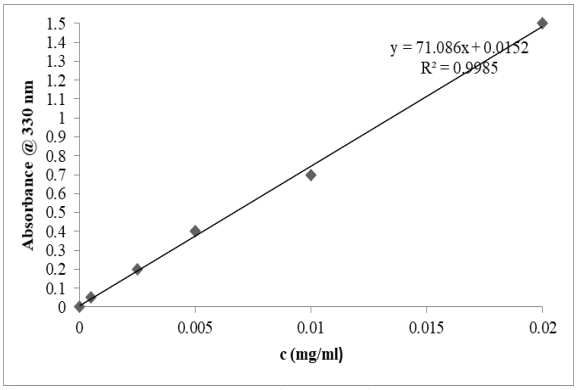

Fig 3: Absorbance values CGA as a function of the solution concentrations. The data correlation was calculated by a linear fit. The tables in the graph report the obtained parameters.

Table 1: Concentration Vs Absorbance at 330 nm table for Linearity Study.

Concentration (mg/ml)	Absorbance @ 330 nm
0.0000	0.000
0.0005	0.053
0.0025	0.210
0.0050	0.460
0.0100	0.750
0.0201	1.500

B. Determination of the Chlorogenic Acids in Green Coffee Bean Extraction.

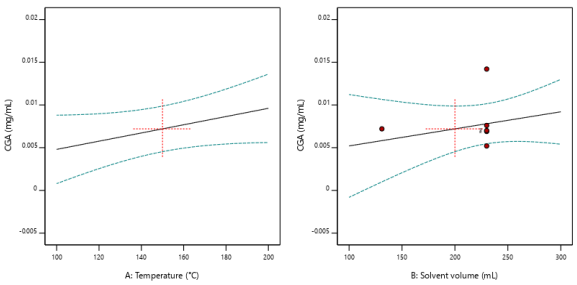

Fig 4: Relationship between CGA concentration(mg/ml) with Temperature (ºC) and Relationship between CGA concentration(mg/ml) with Solvent Volume (ml)

The extracted GCB oils were been diluted before being implemented using the UV- Vis Spectrophotometer. All of GCB oils sample were being diluted with 1:10000 dilution factor to get precise value of absorbance in the range of between the linear fit graphs. Table 2 shows the value of absorbance for every sample of extractions, varies with the amount of solvents and the operating temperature during extraction. From Table 3, it shows that there is significant value of concentration of CGA due to different temperature and solvent volume. In Fig 4, result shows that the CGA concentration will increase linearly to the temperature of the extraction while the solvent volume also will increase linearly to the CGA concentration but will having a few outrange value of solvent value as it indicated that at certain point of solvent volume, there will not follow the linearity of the CGA concentration.

Table 2: Green Coffee Bean Extracted Vs Absorbance at 330 nm

No	Green Coffee Bean Extracted		Absorbance @ 330 nm
	Temperature ($°C$)	Solvent Volume (ml)	
1	100.00	160	0.3651
2	200.00	160	0.6142
3	100.00	300	0.5623
4	200.00	300	1.3141
5	79.29	230	0.1725
6	220.71	230	0.4258
7	150.00	131	0.5267
8	150.00	329	0.4539
9	150.00	230	1.0263
10	150.00	230	0.5108
11	150.00	230	0.5074
12	150.00	230	0.3825
13	150.00	230	0.5587

From the equation of linear fit graph;

$$y = 71.086x + 0.0152$$

Table 3: Green Coffee Bean Extracted Vs Concentration of CGA (mg/ml)

No	Green Coffee Bean Extracted		Concentration of CGA (mg/ml)
	Temperature ($°c$)	Solvent Volume (ml)	
1	100.00	160	25.968
2	200.00	160	43.676
3	100.00	300	39.986
4	200.00	300	93.429
5	79.29	230	12.277
6	220.71	230	30.283
7	150.00	131	37.456
8	150.00	329	32.281
9	150.00	230	72.970

10	150.00	230	36.325
11	150.00	230	36.084
12	150.00	230	27.205
13	150.00	230	39.7309

C. Formulation of Anti-Inflammatory Soap

The saponification value was found to be 396.53 mg/ml. The range of saponification value for coconut oil is 390 mg/ml which complies with the obtained data. The formulated bath soap using GCB extract is shown in Fig 5 with high concentrations that is 0.5% of GCB oils. The extracted sample that had been chosen for the formulation was the optimum range condition of extraction that is 150ºC with 230ml solvent with the range of 75-25 mg/ml of CGA. The physical appearance and other characteristics of the soap were also observed. The soap was totally dried and stable solid bar without any color change. It is foamy in nature when washed hands even without adding any additional foaming agents such as detergents and surfactants. It also has skin compatibility as it didn't show any irritation when tested on ten users. The pH of the soap was determined as 9.5 with pH strip and 9.67 with pH meter. The moisture content of the soap was found to be 6.23 %.

Fig 5: Formulated Green Coffee Bean soap at high concentration (0.5 g) of extract.

D. Anti-Inflammatory Analysis

A. Formalin-induced nociception

At implementation of 0.5g GCB Soap, a significant antinociceptive activity was observed in comparison with the control that is the reference drug, Indometachin, but only during the late phase. However, the analgesic activity of the extracts during the early phase pain was not significant. The reference drug, Indometachin, suppressed only in the second phase of the formalin test. Fig 6 shows that at the first phase of formalin injection, time licking for implementation of 0.5g GCB Soap to the rat were slightly high compare with the reference drug. This may be caused by the diffusion of the CGA into rat paws were slightly slow compare to the direct doses of Indometachin orally. Late at the second phase (Fig 7), it shows that time spend on licking were quite the same with the reference drug, Indometachin. This shown that the CGA from the 0.5g of GCB soap had been diffuse thoroughly the rat paw. Formalin-induced nociception measures the ability of the substance to attenuate moderate continuous pain generated by injured tissue. In the present study, GCB oil produced antinociception only against the inflammatory phase of formalin, suggesting that they may be more effective against inflammatory pain. Drugs that act primarily as central analgesics inhibit both phases, whereas the second phase (inflammatory phase) is characterized by the emergence of a local inflammatory process, where mediators of inflammation are produced. Inhibition of the late phase is due to inflammation causing the release of serotonin, histamine, bradykinin and prostaglandins, which, at least to some degree, can cause sensitization of the central nociceptive neurons [16].

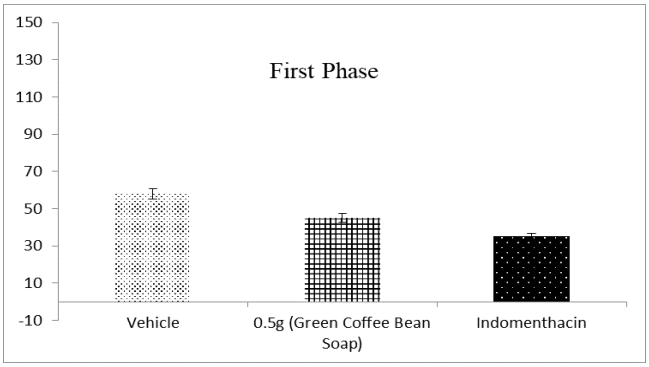

Fig 6: Effects of 0.5g GCB soap, vehicle and Indometachin on the licking induced by formalin in rats for first phase.

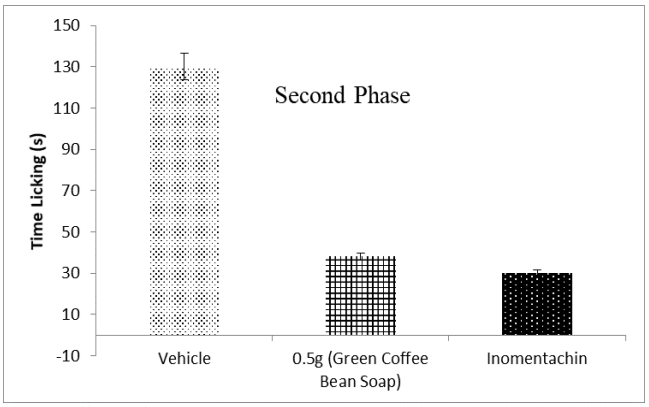

Fig 7: Effects of 0.5g GCB soap, vehicle and Indometachin on the licking induced by formalin in rats for second phase.

B. Carrageenan-induced rat paw oedema

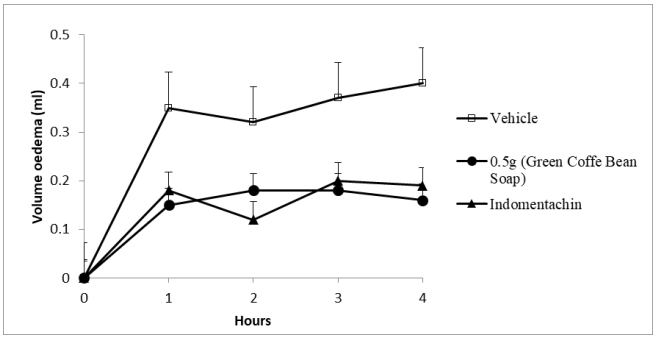

Fig 8: Effects of the administration of 0.5g GCB soap, vehicle and Indometachin

Fig 8 illustrates the significantly inhibited carrageenan-induced rat paw oedema at the third hour. The inhibitory values of oedema at 3 h post carrageenan was 43% for 0.5g GCB soap. This result is quite like that observed for the group treated with Indomethacin, which inhibited oedema formation by 46%. The most widely used primary test for screening anti-inflammatory agents is carrageenan-induced oedema in the rat hind paw [10], which has frequently been used to assess the anti-oedematogenic effect of natural products. After intraplanar injection of carrageenan into rat paws, there are two successive inflammatory phases, followed by a third, no characteristic phase. Within the first hour after carrageenan injection, vascular permeability increases, mediated by histamine and serotonin release; in the second hour, permeability increases as a result of the liberation of kinins and finally, within the third hour, prostaglandins come into action. The treatment of animals with GCB extracts 1 h before carrageenan application also demonstrated mean inhibition at the third hour after stimulus, suggesting another action mechanism derived from arachidonic acid pathways.

A reduction of the late phase behavioral response to a formalin injection was observed, demonstrating the anti-inflammatory activity produced by the soap. The result obtained from the carrageenan-induced rat paw oedema and lipopolysaccharide-induced peritonitis tests also confirmed this effect. These results are in agreement with those of [14] who reported that kahweol, a coffee-specific diterpene, significantly reduced the paw oedema induced by carrageenan and also markedly reduced the level of PGE2 production in the inflamed paw. Verifying our results, Dos Santos demonstrated that chlorogenic acids inhibited carrageenan-induced paw oedema beginning at the second hour of the experimental procedure. CGA also inhibited the number of flinches in the late phase of the formalin-induced pain test. Such activities may be derived from the inhibitory action of CGA in the peripheral synthesis/release of inflammatory mediators involved in these responses [15]

IV. Conclusion

Results reported here suggest that Green Coffee Bean extracts display considerable anti-inflammatory action by alleviating paw oedema, formalin-induced pain test. The mechanism of effect may be due to the presence of anti-inflammatory substances like CGA, flavonoids and antioxidants, which pare present in Green Coffee Bean, as evidenced by previous reports. The production of soap also shows that there was no inflammation for users and usable for any user with best quality. Our result suggests a better anti-inflammatory effect for the extract of green coffee with highest concentration in the soap that is 0.5g of the soap. The extract that will give the most concentrated CGA will be with the operating temperature of between 100ºC-200ºC using 300ml of ethanol as the solvent. In the future, the extracts of Green Coffee Bean may have potential therapeutic value in the treatment of inflammatory disorders.

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